

1 **Influence of dietary nitrate supplementation on physiological and**
2 **muscle metabolic adaptations to sprint interval training**

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12 **Running head:** Dietary nitrate and sprint interval training

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20 **Abstract**

21 We hypothesized that 4 weeks of dietary nitrate supplementation would enhance exercise
22 performance and muscle metabolic adaptations to sprint interval training (SIT). Thirty six
23 recreationally-active subjects, matched on key variables at baseline, completed a series of
24 exercise tests before and following a 4 week period in which they were allocated to one of the
25 following groups: 1) SIT and NO₃⁻-depleted beetroot juice as a placebo (SIT+PL); 2) SIT and
26 NO₃⁻-rich beetroot juice (~13mmol NO₃⁻/day; SIT+BR); or 3) no training and NO₃⁻-rich
27 beetroot juice (NT+BR). During moderate-intensity exercise, pulmonary $\dot{V}O_2$ was reduced by
28 4% following 4 weeks of SIT+BR and NT+BR ($P<0.05$) but not SIT+PL. The peak work rate
29 attained during incremental exercise increased more in SIT+BR than in SIT+PL ($P<0.05$) or
30 NT+BR ($P<0.001$). The reduction in muscle and blood [lactate] and the increase in muscle
31 pH from pre- to post-intervention was greater at 3 min of severe-intensity exercise in
32 SIT+BR compared to SIT+PL and NT+BR ($P<0.05$). However, the change in severe-
33 intensity exercise performance was not different between SIT+BR and SIT+PL ($P>0.05$). The
34 relative proportion of type IIx muscle fibers in the m. vastus lateralis was reduced in SIT+BR
35 only ($P<0.05$). These findings suggest that BR supplementation may enhance some aspects of
36 the physiological adaptations to SIT.

37 **New and Noteworthy:** We investigated the influence of nitrate-rich and nitrate-depleted
38 beetroot juice on the muscle metabolic and physiological adaptations to 4 weeks of sprint
39 interval training. Compared to placebo, dietary nitrate supplementation reduced the O₂ cost of
40 submaximal exercise, resulted in greater improvement in incremental (but not severe-
41 intensity) exercise performance, and augmented some muscle metabolic adaptations to
42 training. Nitrate supplementation may facilitate some of the physiological responses to sprint
43 interval training.

44 **Key words:** beetroot juice supplementation, exercise training, training adaptation, muscle
45 metabolism.

46

47 **Introduction**

48 The gaseous biological signaling molecule, nitric oxide (NO), is known to modulate several
49 physiological responses to exercise including skeletal muscle perfusion, energy metabolism
50 and contractile function (41, 69). Nitric oxide synthase (NOS) enzymes catalyze the oxygen
51 (O_2)-dependent production of NO from L-arginine and it is now known that the products of
52 NO oxidation, nitrate (NO_3^-) and nitrite (NO_2^-), can be reduced *in vivo* to form NO (52, 72).
53 Interestingly, hypoxia and acidosis, physiological environments typical of muscular exercise,
54 facilitate the reduction of NO_2^- to NO (72). Increasing the dietary intake of inorganic NO_3^- to
55 augment circulating NO_3^- and NO_2^- pools may therefore represent a natural means to increase
56 NO bioavailability during exercise.

57 The physiological effects of NO_3^- ingestion in humans are well documented and may include
58 a reduction in blood pressure (BP) at rest and reduced oxygen uptake ($\dot{V}O_2$) during sub-
59 maximal exercise (5, 16, 45, 73). Moreover, several studies suggest that NO_3^-
60 supplementation can improve performance in a variety of exercise settings, at least in sub-
61 elite athletes (2, 14, 71, 73, 78, cf. 40). It has recently been reported that short-term (3-7 days)
62 NO_3^- supplementation may favorably impact the metabolic and contractile properties of
63 skeletal muscle (30, 46, 76). Specifically, the improvements in exercise efficiency and
64 performance that have been observed following dietary NO_3^- supplementation may be related
65 to altered mitochondrial function (46, cf. 76) and to enhanced muscle force or power
66 production (19, 30) which, in turn, might be related to increased perfusion and contractile
67 function (22, 32). It is unclear whether more protracted periods (several weeks) of NO_3^-
68 supplementation may more favorably impact the physiological response to exercise and
69 improve exercise performance. However, given that dietary NO_3^- may specifically enhance
70 the physiological responses of type II muscle fibers to exercise (22, 23, 32, 35), and improve

71 performance during repeated sprint exercise (2, 71, 78), it is possible that NO_3^-
72 supplementation may be of particular value to athletes engaging in high-intensity training.

73 Sprint interval training (SIT) is known to provide a potent and relatively time-efficient
74 stimulus for enhancing aerobic capacity and endurance exercise performance (11, 12, 13, 26).

75 However, the effects of a high- NO_3^- dietary supplement, such as beetroot juice, consumed
76 daily as part of an exercise training program, on the physiological and muscle metabolic
77 adaptations to training has received limited attention (21, 57). It is possible that the NO-
78 mediated inhibition of O_2 consumption at cytochrome c oxidase (10, 17) and resultant local
79 hypoxia may initiate signaling cascades that may be synergistic (or antagonistic) to those
80 generated by SIT (27). Also, similar to the effects of training, elevated NO bioavailability
81 may stimulate angiogenesis (25), mitochondrial biogenesis (58) and the transformation of
82 muscle fiber phenotype (59, 68) through cGMP-dependent gene expression and the activation
83 of regulatory factors, in particular peroxisome proliferator-activated receptor-gamma
84 coactivator-1 alpha (PGC-1 α ; 37, 43). It could also be anticipated that the lower $\dot{\text{V}}\text{O}_2$ and
85 reduced adenosine triphosphate (ATP) and phosphocreatine (PCr) cost of muscle force
86 production during high-intensity exercise following NO_3^- supplementation (6, 24) might
87 enable a higher training intensity for the same effort which, over time, may lead to greater
88 training adaptation (34). An increase in cytosolic calcium concentration ($[\text{Ca}^{2+}]$) and force
89 production during muscle contraction following NO_3^- supplementation (32) may also permit a
90 higher training intensity to be maintained. Given the potentially complementary effects of
91 exercise training and NO bioavailability on metabolic regulation, it is possible that NO_3^-
92 supplementation could augment the physiological adaptations to SIT.

93 Two recent studies have used different approaches to address this question and have
94 produced somewhat disparate results (21, 57). Muggeridge et al. (57) reported that, compared
95 to placebo, NO_3^- supplementation with gels during 3 weeks of SIT (4-6 x 15-s sprints, 3 times

per week) tended to increase peak work rate during incremental exercise (8.7 vs. 4.7 %; $P=0.07$) and reduce the fatigue index during repeated sprint exercise (0.5 vs. 7.3 %; $P=0.06$). De Smet et al. (21) reported that, compared to placebo, NaNO_3 supplementation during 5 weeks of SIT (4-6 x 30-s sprints, 3 times per week), performed in hypoxia, did not improve either incremental exercise or 30-min time trial performance but did result in a significant increase in the proportion of type IIa fibers in the m. vastus lateralis. Neither study measured potential training-related differences in muscle metabolic responses to exercise with NO_3^- compared to placebo supplementation (for example, [PCr], pH, [lactate] and [glycogen] as determined from muscle biopsy) or compared the effects of training with NO_3^- or placebo to the physiological effects of NO_3^- supplementation alone. It would be of interest to determine whether the intriguing change in muscle fiber type proportions when SIT in hypoxia was performed with NO_3^- supplementation (21) is also evident following SIT in normoxia. Additional studies are clearly required to explore the influence of NO_3^- supplementation on the muscle metabolic adaptations and submaximal and maximal exercise responses to training.

The purpose of this study was therefore to evaluate the independent and combined performance and physiological effects of SIT and NO_3^- supplementation during a 4 week intervention involving: SIT with concurrent NO_3^- -depleted beetroot juice supplementation as a placebo (PL); SIT with concurrent NO_3^- -rich beetroot juice supplementation (BR); and NO_3^- -rich beetroot juice supplementation with no training. We tested the hypothesis that 4 weeks SIT and 4 weeks BR supplementation would independently improve physiological responses and exercise performance, but that these effects would be greater when BR supplementation and SIT were combined.

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Methods

Subjects

Eighteen male (mean \pm SD: age 27 ± 8 years, height 1.79 ± 0.08 m, body mass 80 ± 13 kg, $\dot{V}O_{2\text{peak}}$ 50.4 ± 11.4 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) and 18 female (mean \pm SD: age 23 ± 4 years, height 166 ± 5 cm, body mass 65 ± 9 kg, $\dot{V}O_{2\text{peak}}$ 39.8 ± 5.8 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) participants were recruited. The subjects were recreationally-active sportspeople involved in team and/or endurance sports but they were not highly trained. Following an explanation of the experimental procedures, associated risks, potential benefits and likely value of the possible findings, subjects gave their written informed consent to participate. The study was approved by the Institutional Research Ethics Committee and conformed to the code of ethics of the Declaration of Helsinki.

Experimental Design

Subjects initially visited the laboratory on 3 separate occasions over a 5 day period. On visit 1, subjects performed an incremental exercise test on a cycle ergometer for the determination of $\dot{V}O_{2\text{peak}}$ and gas exchange threshold (GET). The work rates requiring 80% of the GET (moderate exercise) and 85% Δ (GET plus 85% of the difference between the work rate at GET and $\dot{V}O_{2\text{peak}}$; severe exercise) were calculated and adjusted for mean response time for $\dot{V}O_2$ during incremental exercise (75). Following this, subjects were familiarized to the exercise testing procedures, including completion of a severe-intensity bout of cycle ergometry until exhaustion. On visit 2, subjects completed a 5-min bout of moderate-intensity cycling and an incremental exercise test. On visit 3, subjects completed 2 bouts of severe-intensity cycling, the first for 3 min and the second until task failure.

In a double-blind, independent-groups design, subjects were then assigned to receive NO_3^- rich beetroot juice (BR) or NO_3^- depleted beetroot juice (PL) for 28 days. Three independent groups ($n = 12$, comprising 6 males and 6 females) were matched at baseline for physical characteristics (i.e. mass, height and age) as well as physiological and performance variables of interest, principally BP and peak WR during incremental exercise and secondarily $\dot{V}\text{O}_{2\text{ peak}}$ and GET. Subjects were then either enrolled onto a 4-week supervised SIT program with PL (SIT+PL) or BR (SIT+BR) supplementation, or received the NO_3^- -rich beetroot juice for 28 days without undergoing a training intervention (NT+BR).

All groups completed the same exercise tests (at the same absolute work rates) and physiological assessments both before and after the 28-day intervention period. Also, after 14 days, subjects visited the laboratory for an incremental exercise test to assess the short-term changes in aerobic capacity that may be expected following the interventions (11, 65, 73).

Laboratory visits were scheduled at the same time of day (± 2 h). Subjects were asked to maintain their normal dietary and exercise behavior throughout the study. However, subjects were instructed to record their diet during the 24 h preceding the first laboratory visit and to repeat this for all subsequent laboratory visits. On days of training, subjects were asked to arrive at the training venue ≥ 1 h post-prandial and to complete a 5 min self-paced warm up before training commenced. On experimental days, subjects were instructed to arrive at the laboratory ≥ 3 h post-prandial having avoided strenuous exercise and the consumption of alcohol and caffeine in the 12 h preceding each exercise test. For the duration of the study, subjects were asked to refrain from taking other dietary supplements, and also to avoid using antibacterial mouthwash as this inhibits the reduction of NO_3^- to NO_2^- in the oral cavity by eliminating commensal bacteria (29).

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Supplementation

Following the pre-intervention laboratory visits, subjects were allocated to receive concentrated NO_3^- -rich beetroot juice (BR; beetroot juice; ~ 6.4 mmol of NO_3^- per 70 mL; Beet it, James White Drinks Ltd., Ipswich, UK) or NO_3^- -depleted beetroot juice (PL; placebo beetroot juice; ~ 0.04 mmol NO_3^- per 70 mL; Beet it, James White Drinks Ltd., Ipswich, UK). Subjects consumed 1 x 70 mL of their allocated supplement each morning and evening for the duration of the training or non-training intervention and recorded their intake in a diary. This approach would be expected to result in elevated plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ for each 24 h period (79). Compliance was checked by the return of empty bottles each week and via questionnaire at 2 and 4 weeks. BR and PL doses were administered using a double blind design. On experimental visits at the mid-intervention point and following the intervention period, subjects consumed 2 x 70 mL of their allocated supplement 2.5 hours prior to the exercise tests.

Incremental exercise tests

On the first laboratory visit before and following the intervention period as well as at the mid-intervention point, subjects completed a ramp incremental exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The self-selected cadence (75-90 rpm), saddle and handle bar height and configuration for each subject were recorded on the first visit and reproduced in subsequent visits. Initially, subjects performed 3 min of baseline cycling at 20 W, after which the work rate was increased by 30 W/min until the limit of tolerance. Breath-by-breath pulmonary gas exchange data (Oxycon Pro, Jaeger, Hoechberg, Germany) were collected continuously throughout all incremental tests and were averaged over 10-s periods. $\dot{\text{V}}\text{O}_{2\text{peak}}$ and GET were determined as previously described (73).

197 *Step exercise tests*

198 A 5-min moderate-intensity “step” test was performed on the first laboratory visit before and
199 following the intervention. This was completed 10 min before the ramp incremental test
200 protocol was initiated. On the second laboratory visit before and following the intervention,
201 two severe-intensity step tests were performed, separated by a 20 min period of rest; the first
202 until 3 min, and the second, after 20 min of passive recovery, until task failure. The time to
203 task failure was recorded once the pedal rate fell by >10 rpm below the target cadence. All
204 step tests began with 3 min of pedaling at 20 W before a sudden transition to the target work
205 rate. Muscle biopsies were obtained before and following the 3-min severe intensity exercise
206 bout and again at task failure in the second bout. Breath-by-breath pulmonary gas exchange
207 data were collected continuously throughout all step tests.

208

209 *Training intervention*

210 Following the initial laboratory visits, subjects were allocated to one of the two SIT groups:
211 SIT with PL supplementation (SIT+PL; age 25 ± 7 years, height 174 ± 10 cm, body mass 73
212 ± 10 kg); SIT with BR supplementation (SIT+BR; mean \pm SD, age 24 ± 7 years, height $174 \pm$
213 11 cm, body mass 78 ± 18 kg); or the non-training group with BR supplementation (NT+BR;
214 age 25 ± 7 years, height 170 ± 6 cm, body mass 68 ± 9 kg). All three groups consisted of 6
215 male and 6 female subjects. Both SIT groups completed a total of 14 supervised training
216 sessions over a 4-week period, with at least 24-h separating each training session, while the
217 NT group maintained their habitual exercise patterns. The post intervention laboratory tests
218 were performed at least 48h following, but within 4 days of, completing the final training
219 session.

220

During the training sessions, the SIT groups completed a series of 30-s “all-out” sprints (i.e. Wingate test) against a resistance equivalent to 7.5% body mass on a mechanically-braked ergometer (model 814E bicycle ergometer, Monark, Stockholm, Sweden; 11, 12, 13). Each sprint was separated by a 4-min period of rest in which subjects cycled at a low cadence against a light resistance to reduce venous pooling and sensations of nausea. During weeks 1 and 2 of training, subjects performed 4 x 30-s sprints three times per week, while during weeks 3 and 4, subjects performed 5 x 30-s sprints four times per week. Following a 5-min warm up of cycling against a light resistance, subjects were given a 10-s count down and instructed to pedal maximally for 2 s before the appropriate load was applied. Subjects were verbally encouraged to maintain maximal cadence throughout each 30-s sprint.

Measurements

Blood pressure and heart rate

Before and following the intervention, as well as at the mid-intervention point, the BP at the brachial artery was measured using an automated sphygmomanometer (Dinamap Pro: GE Medical Systems, Tampa, FL). Following 10 min seated rest in an isolated room, three measurements were recorded. MAP was calculated as $\frac{1}{3}$ systolic pressure + $\frac{2}{3}$ diastolic pressure. The mean of the systolic, diastolic and MAP measurements were used for data analysis.

Blood analysis

Venous blood was sampled at rest (baseline) before each experimental test. Blood samples were also obtained at 1-min, at 3 min and at exhaustion during the severe-intensity exercise bout. The blood samples collected during the severe-intensity exercise bout were drawn from a cannula (Insyte-WTM, Becton Dickinson, Madrid, Spain) inserted into the subject's

antecubital vein and were collected into lithium-heparin vacutainers (Becton Dickinson, New Jersey, USA). Blood [lactate] and [glucose], as well as plasma $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ were analyzed in all samples (square brackets denote concentration). 200 μL of blood was immediately extracted from the lithium-heparin vacutainers and hemolysed in 200 μL of Triton X-100 solution (Triton X-100, Amresco, Salon, OH) before blood [lactate] and [glucose] were measured (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood from each sample was centrifuged at 4000 rpm for 8 min at 4 °C within 2 min of collection. Plasma was immediately extracted, frozen at -80 °C and subsequently analyzed for $[\text{NO}_2^-]$ and $[\text{NO}_3^-]$ using chemiluminescence, as described by Wylie et al. (79).

Muscle biopsy

Muscle samples were obtained from two incisions from the medial region of the *m. vastus lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle biopsy technique (7) with suction. Muscle samples were taken at three different time points before and following the intervention: at rest; following 3 min of severe-intensity exercise; and at task failure from severe-intensity exercise. The post-exercise biopsies were taken while subjects remained on the cycle ergometer and were typically collected within 5-10 s of the completion of the exercise bout. Biopsy samples were immediately frozen in liquid nitrogen and stored at -80 °C for subsequent analysis.

Muscle metabolites

Following a freeze-drying process, samples were dissected to remove visible blood, fat, and connective tissue. Approximately 2 mg aliquots of isolated muscle fibers were weighed on fine balance scales (Mettler Toledo XS105, Leicester, UK) and stored in 500 μL

microcentrifuge tubes at -80 °C. Prior to metabolite analysis, 200 µL of 3 M perchloric acid was added to ~2 mg dry weight muscle tissue. Following 3 min centrifugation and 30 min incubation on ice, 170 µL of supernatant was transferred to a fresh microcentrifuge tube and 255 µL of cooled 2 M potassium bicarbonate (KHCO₃) was added. This was centrifuged, and the supernatant analyzed for [PCr], [ATP] and [lactate] by fluorometric assays as previously described (51).

Muscle glycogen and pH

Glycogen was extracted from ~1 mg d.w. muscle in 500 µL of 1 M hydrochloric acid (HCl) and hydrolyzed at 100 °C for 2 h to glycosyl units, which were measured using an automated glucose analyser (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH) to determine muscle [glycogen]. Muscle pH was measured using a micro-pH meter (Sentron SI600, Roden, The Netherlands) following homogenization of ~1 mg d.w. muscle in a non-buffering solution (145 mM KCl, 10 mM NaCl and 5 mM NaF).

Muscle fiber type

Approximately 20 mg of tissue obtained from each resting muscle biopsy sample was embedded in Tissue-Tek® O.T.C.™ compound (Sakura Finetek Europe BV Zoeterwoude, The Netherlands), rapidly frozen in liquid nitrogen-cooled isopentane, and stored at -80 °C for subsequent histochemical analysis of myocellular characteristics. Serial cross sections (~10 µM thick) were cut in a cryostat (Cryostar NX50, Thermo Scientific, USA) maintained at -16 °C. Sections were mounted on 3 separate slides and pre-incubated at pH values of 4.3, 4.6 and 10.3. According to the lability to the acid and alkaline pre-incubation, the fibers were stained for myofibrillar ATPase, identified as type I, IIa, or IIx (9) and counted under an Olympus CKX41 microscope with cellSens Dimension software (Olympus Corporation,

Tokyo, Japan). For each subject, 214 (\pm 104) fibers were analyzed, and each fiber type was expressed as a percentage of the total number counted.

Oxygen uptake. The breath-by-breath $\dot{V}O_2$ data from each step exercise test were initially examined to exclude values lying more than four SDs from the local mean. The filtered data were subsequently linearly interpolated to provide second-by-second values and time-aligned to the start of exercise for each individual. The baseline $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 60 s of the 3 min baseline period. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 60 s of exercise.

Statistical analyses

Differences between groups in pre-intervention physiological and performance values were tested using a one way ANOVA. Time by group ANOVAs with repeated measures for time were employed to determine the physiological and performance effects consequent to the interventions. In addition, one-way ANOVAs were used to assess differences between groups in the change values for physiological and performance variables pre- to post-intervention. All significant main and interaction effects were followed up by Fisher's LSD post hoc tests. Data that were not normally distributed were log transformed before applying the ANOVA. All values are reported as mean \pm SD. Statistical significance was accepted at $P < 0.05$

Results

Compliance

All subjects within the training groups completed 100% of the training sessions and 100% of the sprints within each training session. All subjects reported that they fully adhered to the

319 supplementation regimen and did not alter dietary and exercise behavior outside of their
320 assigned group-specific intervention.

321

322 Plasma $[NO_3^-]$ and $[NO_2^-]$

323 Pre-intervention resting plasma $[NO_3^-]$ values were not different between groups ($P>0.05$). A
324 significant main effect for time ($P<0.001$) and an interaction effect ($P<0.001$) was observed
325 for the plasma $[NO_3^-]$ measured at rest. Compared to pre-intervention, SIT+BR increased
326 resting plasma $[NO_3^-]$ by ~590% at 2 weeks and ~960% at 4 weeks (both $P<0.001$; Fig. 1A)
327 and NT+BR increased resting plasma $[NO_3^-]$ by ~505% at 2 weeks and ~1050% at 4 weeks
328 (both $P<0.001$; Fig. 1A) but there was no change in resting plasma $[NO_3^-]$ with SIT+PL
329 ($P>0.05$; Fig. 1A). Resting plasma $[NO_3^-]$ was also greater at 4 weeks compared to 2 weeks
330 in both SIT+BR and NT+BR ($P<0.05$; Fig. 1A).

331 Pre-intervention resting plasma $[NO_2^-]$ values were higher in SIT+PL (74 ± 62 nM) compared
332 to SIT+BR (29 ± 19 nM; $P<0.05$) and NT+BR (26 ± 13 nM; $P<0.05$) but were similar
333 between SIT+BR and NT+BR ($P>0.05$). There was a significant main effect for time
334 ($P<0.001$) and an interaction effect ($P<0.001$) for the plasma $[NO_2^-]$ measured at rest.
335 Compared to pre-intervention, SIT+BR increased resting plasma $[NO_2^-]$ by ~485% at 2
336 weeks and ~715% at 4 weeks (both $P<0.001$; Fig. 1B) and NT+BR increased resting plasma
337 $[NO_2^-]$ by ~600% at 2 weeks and ~690% at 4 weeks (both $P<0.001$; Fig. 1B) but there was no
338 change in resting plasma $[NO_2^-]$ with SIT+PL ($P>0.05$; Fig. 1B). There were no differences
339 in the plasma $[NO_2^-]$ measured at rest between 2 weeks and 4 weeks in any of the groups
340 ($P>0.05$).

341 Blood Pressure

342 Systolic BP was not different between the groups before the interventions ($P>0.05$; Table 1)
343 but there was a significant main effect for time ($P<0.05$) and an interaction effect ($P<0.05$).
344 Post hoc tests revealed that, compared to pre-intervention, systolic BP was reduced at 2
345 weeks and at 4 weeks ($P<0.05$) by 5 ± 6 mmHg and 6 ± 4 mmHg in SIT+BR, respectively

($P<0.05$), and by 4 ± 5 mmHg and 10 ± 6 mmHg in NT+BR, respectively ($P<0.05$), whereas systolic BP remained unaltered in SIT+PL ($P>0.05$; Table 1). Diastolic BP was not different between groups at pre-intervention ($P>0.05$) and remained unaltered at 2 weeks and at 4 weeks (both $P>0.05$) in all interventions (Table 1). The MAP was not different between groups at pre-intervention but there was a significant main effect for time ($P<0.05$) such that MAP was reduced by 3 ± 5 mmHg at 4 weeks in both SIT+BR and NT+BR ($P<0.05$) but was unchanged with SIT+PL (Table 1). Relative to post-intervention resting baseline, plasma $[\text{NO}_2^-]$ declined by $\sim 65\%$ at task failure during severe-intensity exercise ($P<0.001$) in SIT+BR and NT+BR. The reduction in plasma $[\text{NO}_2^-]$ following 3 min of severe-intensity exercise was greater in NT+BR compared to SIT+BR ($P<0.05$).

Incremental exercise test

Peak WR was not different between the groups at pre-intervention ($P>0.05$; Table 1). There was a significant main effect by time ($P<0.001$) and an interaction effect ($P<0.05$). Post hoc tests revealed that peak WR was improved at 4 weeks compared to pre-intervention in all groups ($P<0.05$; Table 1). However, peak WR increased more from pre- to post-intervention in SIT+BR than in SIT+PL ($P<0.001$; Fig. 2). Additionally, peak WR was improved at 2 weeks compared to pre-intervention in SIT+BR only ($P<0.05$; Fig. 2).

$\dot{V}\text{O}_{2\text{peak}}$ was not different between the groups at pre-intervention ($P>0.05$; Table 1). There was a significant main effect by time on $\dot{V}\text{O}_{2\text{peak}}$ ($P<0.05$). Post hoc analysis revealed that, compared to pre-intervention, $\dot{V}\text{O}_{2\text{peak}}$ was increased after 2 weeks and 4 weeks with SIT+BR ($P<0.05$; Table 1) but remained unchanged in SIT+PL and NT+BR ($P>0.05$; Table 1). However, there were no differences between the three groups in the change in $\dot{V}\text{O}_{2\text{peak}}$ from pre- to post-intervention ($P>0.05$). There were no significant changes in body mass from pre- to post-intervention in any of the groups.

The $\dot{V}O_2$ at the GET was not different between the groups at pre-intervention ($P>0.05$; Table 1) and was not altered by any intervention ($P>0.05$; Table 1). The WR associated with the GET was not different between groups at pre-intervention ($P>0.05$; Table 1). There was a significant main effect for time such that the WR at the GET was increased pre- to post-intervention in SIT+BR only ($P<0.05$; Table 1). However, there were no differences between the three groups in the change in the WR at the GET from pre- to post-intervention ($P>0.05$).

Step exercise tests: Moderate-intensity exercise

The $\dot{V}O_2$ measured during baseline cycling at 20 W preceding the transition to moderate-intensity exercise was not different between groups at pre-intervention ($P>0.05$; Table 1) and was not affected by any intervention ($P>0.05$; Table 1). The end-exercise $\dot{V}O_2$ during moderate-intensity exercise was not different between groups at pre-intervention ($P>0.05$; Table 1). There was a significant main effect by time ($P<0.05$) and an interaction effect ($P<0.05$) on end-exercise $\dot{V}O_2$. Post hoc analyses revealed that, compared to pre-intervention, end-exercise $\dot{V}O_2$ was significantly reduced in SIT+BR ($P<0.05$) and NT+BR ($P<0.05$) but was unaltered in SIT+PL ($P>0.05$; Table 1). There was no difference in the change in end-exercise $\dot{V}O_2$ from pre- to post-intervention between the SIT+BR and NT+BR groups ($P>0.05$).

Step exercise tests: Severe-intensity exercise

The time to task failure during severe-intensity exercise was not different between groups at pre-intervention ($P>0.05$; Table 1). There was a significant main effect by time ($P<0.05$) and an interaction effect ($P<0.05$) such that time to task failure was improved by 163 ± 144 s pre- to post- intervention in SIT+PL ($P<0.05$; Table 1) and by 170 ± 90 s pre- to post-intervention in SIT+BR ($P<0.05$; Table 1) but was unaltered by NT+BR ($P>0.05$; Table 1). There was no

difference in the change in the time to task failure from pre- to post-intervention between the SIT+BR and SIT+PL groups ($P>0.05$).

Blood [lactate] was not different between groups during severe-intensity exercise at pre-intervention ($P>0.05$). There was a main effect by time on blood [lactate] ($P<0.05$). Post-hoc analysis revealed that blood [lactate] was lower at 1 min (1.2 ± 1.1 mM decrease from same time point pre-intervention; $P<0.05$; Fig. 3) and at 3 min (1.6 ± 1.5 mM decrease from same time point pre-intervention; $P<0.05$, Fig. 3) during severe-intensity exercise in SIT+BR but not SIT+PL or NT+BR ($P>0.05$). Further analyses revealed that the increase in blood [lactate] from rest to 3 min was attenuated post-intervention compared to pre-intervention in SIT+BR (2.7 ± 0.9 vs. 3.9 ± 0.8 mM) ($P<0.05$). This attenuation was significantly greater than the equivalent change in blood [lactate] from rest to 3 min in SIT+PL (post-intervention: 4.1 ± 1.9 vs. pre-intervention: 3.7 ± 1.2 mM; $P<0.05$).

Muscle substrates and metabolites

Pre-intervention values for muscle substrates and metabolites during severe-intensity exercise were not different between groups ($P>0.05$) and muscle [ATP] and [PCr] were unchanged by the interventions in all groups ($P>0.05$).

There were main effects by time on the muscle [lactate] and pH measured at 3 min of severe-intensity exercise ($P<0.05$). Post hoc tests revealed that, compared to pre-intervention, muscle [lactate] was lower and pH was higher at 3 min of severe-intensity exercise post-intervention in SIT+BR ($P<0.05$; Fig. 4). Further analyses revealed that, compared to pre-intervention, the increase in muscle [lactate] and the decrease in muscle pH from rest to 3 min of exercise tended to be attenuated post-intervention in SIT+ BR (both $P=0.09$).

415 There was a main effect by time on muscle [glycogen] measured at rest, at 3 min and at
416 exhaustion (all $P<0.05$). Post hoc tests revealed that, compared to pre-intervention, muscle
417 [glycogen] was higher at all three time points post-intervention compared to pre-intervention
418 in SIT+BR ($P<0.05$; Fig 5). Muscle [glycogen] was also higher at all three time points post-
419 intervention in SIT+BR and SIT+PL compared to NT+BR ($P<0.05$; Fig 4). There were no
420 differences between SIT+BR and SIT+PL in the change in muscle [glycogen] from pre- to
421 post- intervention at rest, 3 min of exercise or at exhaustion ($P>0.05$).

422 *Muscle fiber type*

423 The relative proportion of type I (SIT+BR: $57 \pm 16\%$; SIT+PL: $59 \pm 10\%$; NT+BR: $48 \pm$
424 16%), type IIa (SIT+BR: $36 \pm 12\%$; SIT+PL: $36 \pm 16\%$; NT+BR: $44 \pm 16\%$) and type IIx
425 (SIT+BR: $7 \pm 8\%$; SIT+PL: $5 \pm 7\%$; NT+BR: $8 \pm 12\%$) muscle fibers at pre-intervention
426 were not different between groups ($P>0.05$). There was a significant effect of time and an
427 interaction effect on the proportion of type IIx fibers. Post hoc tests revealed that the
428 proportion of type IIx fibers identified in SIT+BR was lower post-intervention ($4 \pm 5\%$)
429 compared to pre-intervention ($7 \pm 8\%$; $P<0.05$). In contrast, the proportion of type IIx fibers
430 identified in SIT+PL tended to be higher post-intervention ($10 \pm 9\%$) compared to pre-
431 intervention ($5 \pm 7\%$; $P=0.07$). The change in type IIx fibers was significantly different in
432 SIT+BR compared to SIT+PL ($P<0.05$) but not NT+BR ($P>0.05$). There were no differences
433 in the proportion of type I (SIT+BR: $55 \pm 12\%$; SIT+PL: $58 \pm 10\%$; NT+BR: $50 \pm 17\%$) or
434 type IIa (SIT+BR: $41 \pm 9\%$; SIT+PL: $32 \pm 16\%$; NT+BR: $43 \pm 14\%$) muscle fibers following
435 any intervention ($P>0.05$). However, there was a significant interaction effect on the
436 proportion of type I and type IIa fibers combined (type I+IIa; $P<0.05$). Post hoc tests revealed
437 that the proportion of type I+IIa fibers identified in SIT+BR was higher post-intervention (96
438 $\pm 6\%$) compared to pre-intervention ($93 \pm 8\%$; $P<0.05$). In contrast, the proportion of type
439 I+IIa fibers identified in SIT+PL tended to be lower post-intervention ($90 \pm 9\%$) compared to

440 pre-intervention ($95 \pm 7\%$; $P=0.07$). The change in type I+IIa fibers was significantly
441 different in SIT+BR and NT+BR compared to SIT+PL ($P<0.05$).

442

443 Discussion

444 This is the first study to investigate the combined effect of SIT and NO_3^- supplementation,
445 administered in the form of beetroot juice, on muscle metabolic adaptations and the
446 physiological responses to ramp incremental, moderate-intensity and severe-intensity
447 exercise performance in normoxia. We compared the effects of chronic NO_3^- supplementation
448 alone (NT+BR) with the effects of concurrent NO_3^- -rich (SIT+BR) and NO_3^- -depleted
449 (SIT+PL) beetroot juice supplementation during a SIT intervention. Consistent with our
450 hypotheses, the separate 4 week interventions of SIT and chronic BR supplementation
451 independently induced several beneficial physiological and/or performance effects. However,
452 the main finding of the present study was that the combination of SIT and BR
453 supplementation provided greater improvements in incremental exercise performance
454 compared to either intervention alone and led to greater improvements in some indices of
455 muscle metabolic adaptation.

456 Plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were elevated, and systolic BP was lowered following 2 weeks and
457 4 weeks of BR supplementation, changes which are consistent with elevated systemic NO
458 bioavailability. Interestingly, however, resting plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were not altered
459 following 4 weeks of SIT+PL. Previous studies have reported that subjects with higher
460 aerobic fitness and/or training status have higher resting plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ compared
461 to less fit and/or sedentary subjects (53, 63). The results of the present study may therefore
462 indicate that short-term SIT, at least when combined with PL supplementation, does not
463 substantially modify NOS activity or protein expression. The reduction in plasma $[\text{NO}_2^-]$
464 from resting baseline to task failure during severe-intensity exercise was similar between
465 NT+BR and SIT+BR (~65% decline). However, the reduction in plasma $[\text{NO}_2^-]$ from resting
466 baseline to 3 min of severe-intensity exercise was attenuated in SIT+BR (~25% decline)
467 compared to NT+BR (~45% decline). It is possible that this may be related to differences in

training status induced by the separate interventions; for example, less reduction of NO_2^- to NO may have been required following SIT+BR due to training-related improvements in muscle capillarity and oxygenation (18).

The reductions in systolic BP (SIT+ BR: -4% and -5%, NT+BR: -6% and -9%, at 2 weeks and 4 weeks, respectively) reported in the present study are similar to those previously reported in healthy volunteers following shorter supplementation periods (5, 44, 73, 74). Diastolic BP was unaltered in NT+BR and SIT+PL but was reduced by 7% in SIT+BR. MAP was lowered by ~4% in both NO_3^- supplemented groups (SIT+BR and NT+BR), but was unaltered in SIT+PL. Collectively, these data indicate that 4 weeks of NO_3^- supplementation may result in a greater reduction in BP than 4 weeks of SIT alone.

The effect of SIT and BR on sub-maximal $\dot{V}\text{O}_2$ and $\dot{V}\text{O}_{2\text{peak}}$

A high exercise economy, i.e. a low $\dot{V}\text{O}_2$ for a given power output, is an important determinant of exercise performance (33). It has been postulated that exercise training can lower the O_2 cost of submaximal cycling (55). However, in the present study, the O_2 cost of moderate-intensity exercise was only reduced following training in SIT+BR and the magnitude of the reduction in the O_2 cost of exercise was not different to that observed with NT+BR, suggesting that 4 weeks of SIT *per se* has no influence on the O_2 cost of submaximal cycling. This finding is consistent with previous work indicating that the O_2 cost of exercise may be reduced by dietary NO_3^- supplementation (5, 45, 46, 73). The physiological bases for the improved efficiency following NO_3^- ingestion are likely related to a reduced ATP cost of muscle force production (6) and/or a reduced O_2 cost of mitochondrial ATP resynthesis (46, cf. 76).

Despite the low training volume, SIT has emerged as a potent strategy to increase aerobic capacity and endurance exercise performance in as little as two weeks (11, 65, 70). We found

that $\dot{V}O_{2peak}$ was not significantly altered by 4 weeks of either NT+BR or SIT+PL. The former result is consistent with the majority of studies that have assessed $\dot{V}O_{2peak}$ following acute or short-term NO_3^- supplementation (5, 6, 39, 45, 78). The lack of effect of SIT on $\dot{V}O_{2peak}$ is also consistent with some (11, 12, 13, 26), but not all (65, 70), previous investigations. The physiological and muscle metabolic adaptations to SIT are likely dependent upon the initial training status of the subjects along with the exact nature of the training stimulus, including the frequency and duration of both the sprint and recovery periods (66). In this respect, it is important to highlight that our exercise training protocol was shorter in duration to some studies (13) and the progression in training volume was more gradual than in other studies (4, 65) in which $\dot{V}O_{2peak}$ was increased.

Although the change in $\dot{V}O_{2peak}$ from pre- to post-intervention was not different between the three groups, the increase in $\dot{V}O_{2peak}$ was only greater from pre- to post intervention in SIT+BR, suggesting that NO_3^- supplementation may enhance the adaptation of $\dot{V}O_{2peak}$ to SIT. Further work is required to confirm this observation and to elucidate the potential cardiovascular and/or metabolic mechanisms which may be responsible.

The effect of SIT and BR on exercise performance

The peak WR during incremental exercise at 4 weeks was improved in both training groups. Interestingly, peak WR was also significantly improved following NT+BR. Although this effect was small, it is consistent with an earlier study which reported a significant increase in peak WR during incremental exercise following 15 days BR supplementation (73). Interestingly, a greater peak WR at 2 weeks of training was only observed with SIT+BR. Moreover, the improvement in peak WR at 4 weeks was greater in SIT+BR than in SIT+PL and NT+BR. The greater, and more rapidly attained, improvements in incremental exercise test performance with SIT+BR is presumably a function of the improved exercise economy

and/or favorable muscle metabolic profile which would be expected to result in an extended time to reach $\dot{V}O_{2peak}$. Our results are consistent with a recent study by Muggeridge et al. (57) which reported that 3 weeks of SIT (4-6 repeated 15-s sprints) increased peak WR during incremental exercise to a greater extent when subjects were supplemented with NO_3^- compared to placebo.

The time to task failure during severe-intensity exercise was significantly increased after 4 weeks of both SIT+BR (group mean change: +69%) and SIT+PL (+55%), but not NT+BR (+3%). Despite evidence for an enhanced muscle metabolic response to severe-intensity exercise in SIT+BR compared to SIT+PL (see below), this did not translate into a greater improvement in severe-intensity exercise performance. It is not clear why this was the case nor why ramp incremental exercise test performance was improved with SIT+BR when time to task failure during severe-intensity exercise was not, although greater variability in time-to-exhaustion tests may have contributed to the difference (20). Indeed, it is interesting to note that the improvement in time to task failure ranged from 37-116% in SIT+BR (with 9/12 subjects improving by more than 50%) and from 4-122% in SIT+PL (with 4/12 subjects improving by more than 50%). Our results are similar to those of Puype et al. (64) who found that 6 weeks of endurance training in normobaric hypoxia with BR supplementation did not improve 30-min time trial performance relative to the placebo condition. However, it remains unclear whether BR supplementation during training could improve performance in other types of exercise. Recent studies indicate that BR may be ergogenic during high-intensity intermittent exercise (2, 71, 78) and that, compared to placebo, NO_3^- supplementation during SIT improves fatigue resistance during repeated sprint exercise (57) and may enhance mean power output in a 30 s sprint (21). Further studies are required to investigate whether the subtle enhancements of skeletal muscle adaptation to training with BR might translate into improved performance during these other forms of exercise.

542 *The effect of SIT and BR on the muscle metabolic response to exercise*

543 Although conflicting data exist, SIT has been implicated in rapid skeletal muscle remodeling
544 (11, 12, 13, 26). The extreme perturbations in substrate availability and metabolite
545 accumulation caused by repeated sprint efforts require substantial oxidative energy turnover
546 to restore homeostasis (8). The fluctuations in ATP availability and local O₂ tension are
547 potent stimulators of signaling pathways and may induce mitochondrial biogenesis and
548 oxidative enzyme adaptation via the transcription of PGC-1 α (28, 31, 50). Recent findings
549 indicate that dietary NO₃⁻ may favorably affect the contractility (30, 32) and perfusion (22,
550 23) of type II muscle fibers, and reduce the energetic cost of muscle force production during
551 high-intensity exercise (6, 24). Similar to SIT (3, 13, 47, 48, 49, 61, 62), elevating NO₂⁻ and
552 NO bioavailability with chronic NO₃⁻-rich BR supplementation may also stimulate the
553 transcription of PGC-1 α (43, 54, 58), a key regulator of mitochondrial biogenesis (77) and
554 angiogenesis (1, 15). We therefore determined the effects of 4 weeks SIT and 4 weeks BR
555 supplementation on the muscle metabolic responses during exercise and tested the hypothesis
556 that these adaptations may be amplified when the interventions were combined.

557 There were no differences in muscle [ATP], [PCr], [lactate] or pH at rest or at task failure
558 during severe-intensity exercise, post-intervention compared to pre-intervention, in any
559 group. However, at 3 min into severe-intensity exercise, there was evidence of reduced
560 metabolic perturbation, post-intervention compared to pre-intervention, in the SIT+BR group
561 only. Specifically, muscle [lactate] as well as blood [lactate] was lower, and muscle pH was
562 higher, at 3 min of severe-intensity exercise following SIT+BR but not SIT+PL or NT+BR
563 (Figs. 3 and 4), suggesting an enhanced muscle metabolic adaptation to SIT when combined
564 with BR supplementation.

The reason for the small difference in muscle acidosis at the 3 min exercise iso-time with SIT+BR compared to SIT+PL is unclear. However, this may be the result of differences in exercise efficiency between the training groups. The lower O₂ cost of exercise measured at the same submaximal work rate in SIT+BR would be expected to lower the physiological strain and potentially reduce substrate-level phosphorylation and lactate production during exercise (34). Furthermore, BR supplementation has been shown to elevate microvascular PO₂ in type II muscles of exercising rats thus promoting O₂ exchange between the capillary and the myocyte and enabling a better preservation of intramuscular homeostasis (22, 23). By better maintaining oxidative function, this mechanism may be important in delaying lactate accumulation during severe-intensity exercise which is known to mandate an increased recruitment of type II fibers to sustain power output (42). While NO₃⁻ intake alone would be expected to promote some of these effects (for example, a lower O₂ cost of sub-maximal exercise in the NT+BR group in the present study), NO₃⁻ intake combined with training may synergistically improve the muscle metabolic response to severe-intensity exercise. In particular, the SIT+BR group evidenced improved exercise efficiency (which was observed with NT+BR but not SIT+PL) and improved performance and physiological responses/adaptations to maximal exercise (which were observed with SIT+PL but to a much lesser extent with NT+BR).

None of the interventions influenced the proportion of type I muscle fibers identified following training. Interestingly, there was a disparity in the muscle phenotypic response to training between SIT+BR and SIT+PL. Specifically, SIT+BR resulted in a significant reduction in the proportion of type IIx muscle fibers. In contrast, SIT+PL resulted in a trend towards a greater proportion of type IIx fibers following the intervention period. These results suggest that a remodeling of skeletal muscle towards a more oxidative phenotype following SIT (26, 27) may be facilitated by BR supplementation and perhaps hampered by PL

supplementation. Our findings are consistent with a recent study which also reported changes in muscle fiber type composition following 5 weeks of SIT with ~5 mmol daily NO_3^- supplementation (21). These authors reported that SIT performed in hypoxia resulted in a significant increase in the relative number of type IIa fibers in the m. vastus lateralis (from ~45 to 56%) when subjects ingested NO_3^- compared to placebo. It is possible that the differences in the muscle metabolic or performance response to exercise following SIT when combined with NO_3^- compared to placebo supplementation (present study; 57) are related to changes in muscle fiber type composition – i.e., a greater reduction in type IIx fibers and/or a greater increase in type IIa fibers (present study; 21).

It is important to highlight that both the BR and PL supplements contain high concentrations of antioxidants including betacyanins and polyphenols (38, 67) which may potentially interfere with skeletal muscle adaptations to training (56, 60). It is possible, therefore, that the adaptations to training in the SIT+PL group were attenuated in the present study due to the simultaneous intake of antioxidants. However, it is also possible that the potential for chronic NO_3^- administration to enhance muscular adaptations and exercise performance with SIT was underestimated in the SIT+BR group for the same reason. On the other hand, it has recently been reported that BR supplementation increases hydrogen peroxide emission from the mitochondria, an effect that could promote redox signaling (76) and enhance training adaptations. Moreover, the combination of NO_3^- with antioxidants might promote the reduction of NO_2^- to NO and facilitate physiological effects (36). Further research should investigate the influence of NO_3^- alone (as NaNO_3 or KNO_3) and BR on the skeletal muscle adaptations to training. Our study design involved 4 weeks of daily BR supplementation with the final dose being consumed on the morning of the post-intervention laboratory tests. Our measurements therefore reflect the combined effects of chronic and acute BR (or PL) supplementation superimposed on exercise training. It has been reported recently that 4

weeks of BR supplementation continues to exert physiological effects for at least 48 hours following the cessation of supplementation (80). Future studies might therefore be designed to partition out the influence of chronic NO_3^- or BR supplementation (without additional acute supplementation) on the adaptations to training.

Conclusions

In the absence of training, chronic BR ingestion resulted in a significant reduction in the O_2 cost of moderate-intensity exercise and a small but significant increase in peak WR. SIT+PL resulted in improvements in peak WR during incremental exercise and time to task failure during severe-intensity exercise. Greater changes in peak WR during incremental exercise were found with SIT+BR compared to SIT+PL and NT+BR. In addition, type IIX muscle fiber proportion was reduced and, at the 3-min iso-time during severe-intensity exercise, muscle pH was higher and muscle (and blood) [lactate] was lower in SIT+BR only. These findings suggest that the independent physiological and performance effects of SIT and BR supplementation may be enhanced when these interventions are combined. Dietary NO_3^- supplementation in the form of BR may potentiate some exercise performance and muscle metabolic adaptations to SIT.

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637

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642

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Figure Legends

Figure 1. Mean \pm SD resting plasma $[\text{NO}_3^-]$ (panel A) and plasma $[\text{NO}_2^-]$ (panel B) responses in SIT+BR (solid black line), SIT+PL (solid grey line) and NT+BR (dotted black line). * = different from pre-intervention ($P<0.05$); † = different from mid-intervention ($P<0.05$); ‡ = different from SIT+PL ($P<0.05$).

Figure 2. Mean \pm SD changes (Δ) in peak WR at mid- and post-intervention in the three groups expressed relative to pre-intervention baseline. The change in peak WR from pre- to post-intervention was greater in SIT+BR (solid black line) than SIT+PL (solid grey line) and NT+BR (dotted black line). * = different from pre-intervention ($P<0.05$), † = different from mid-intervention ($P<0.05$), # = different from NT+BR ($P<0.05$), ‡ = different from SIT+PL ($P<0.05$).

Figure 3. Mean \pm SD blood [lactate] at rest (black bars), 1 min (patterned bars), 3 min (grey bars) and at task failure (open bars) during severe-intensity exercise. * = different to pre-intervention ($P<0.05$).

Figure 4. Mean \pm SD muscle [lactate] (panel A), muscle pH (panel B) and muscle [glycogen] (panel C) at rest (black bars), 3 min (grey bars) and at task failure (open bars) during severe-intensity exercise. * = different to pre-intervention ($P<0.05$); # = different to post-intervention NT+BR ($P<0.05$); ‡ = different to post-intervention SIT+PL ($P<0.05$).

Table 1. Physiological and performance variables pre-, mid- and post-intervention

	SIT+PL			SIT+BR			NT+BR		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
Blood pressure									
SBP (mmHg)	116 ± 13	115 ± 14	116 ± 10	118 ± 11	113 ± 11*	112 ± 10*‡	117 ± 13	113 ± 10*	107 ± 17*‡
DBP (mmHg)	67 ± 8	64 ± 7	66 ± 4	67 ± 9	64 ± 9	62 ± 7	63 ± 5	63 ± 7	62 ± 8
MAP (mmHg)	83 ± 8	81 ± 8	83 ± 6	84 ± 8	80 ± 9	79 ± 7*	82 ± 7	80 ± 7	77 ± 7*
Incremental test									
Peak WR (W)	303 ± 78	306 ± 72	318 ± 73*†	298 ± 93	305 ± 90*	321 ± 91*†	296 ± 66	295 ± 67	300 ± 67*
Δ Peak WR (W)	-	4 ± 13	16 ± 15*†#	-	7 ± 10*#	24 ± 8*†#‡	-	0 ± 9	4 ± 4*
$\dot{V}O_{2\text{ peak}}$ (L·min ⁻¹)	3.43 ± 0.99	3.49 ± 0.97	3.50 ± 0.86	3.19 ± 1.03	3.39 ± 1.06*	3.47 ± 1.02*	3.28 ± 1.03	3.42 ± 0.99	3.42 ± 1.08
$\dot{V}O_2$ at GET (L·min ⁻¹)	1.55 ± 0.49	1.49 ± 0.41	1.62 ± 0.44	1.60 ± 0.37	1.58 ± 0.37	1.64 ± 0.43	1.61 ± 0.46	1.62 ± 0.52	1.61 ± 0.4
WR at GET (W)	110 ± 32	103 ± 34	112 ± 27	102 ± 30	105 ± 32	110 ± 27*	105 ± 34	102 ± 29	112 ± 27

Moderate-intensity exercise									
End-exercise $\dot{V}O_2$ (L·min ⁻¹)	1.57 ± 0.41	-	1.67 ± 0.44	1.64 ± 0.41	-	1.58 ± 0.42*‡	1.73 ± 0.32	-	1.65 ± 0.34*‡
Severe-intensity exercise									
Time to task failure (s)	297 ± 69	-	460 ± 186*#	248 ± 53	-	418 ± 132*#	266 ± 82	-	275 ± 84

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2\text{ peak}}$, peak $\dot{V}O_2$; WR, work rate; GET, gas exchange threshold; SIT+BR, high-intensity interval training plus NO₃⁻-rich beetroot juice; SIT+PL, high-intensity interval training plus NO₃⁻-depleted juice; NT+BR, no-training plus NO₃⁻-rich beetroot juice. * = different from Pre-intervention ($P<0.05$), † = different from Mid-intervention ($P<0.05$), # = different from NT+BR ($P<0.05$), ‡ = different from SIT+PL ($P<0.05$).

Figure 1

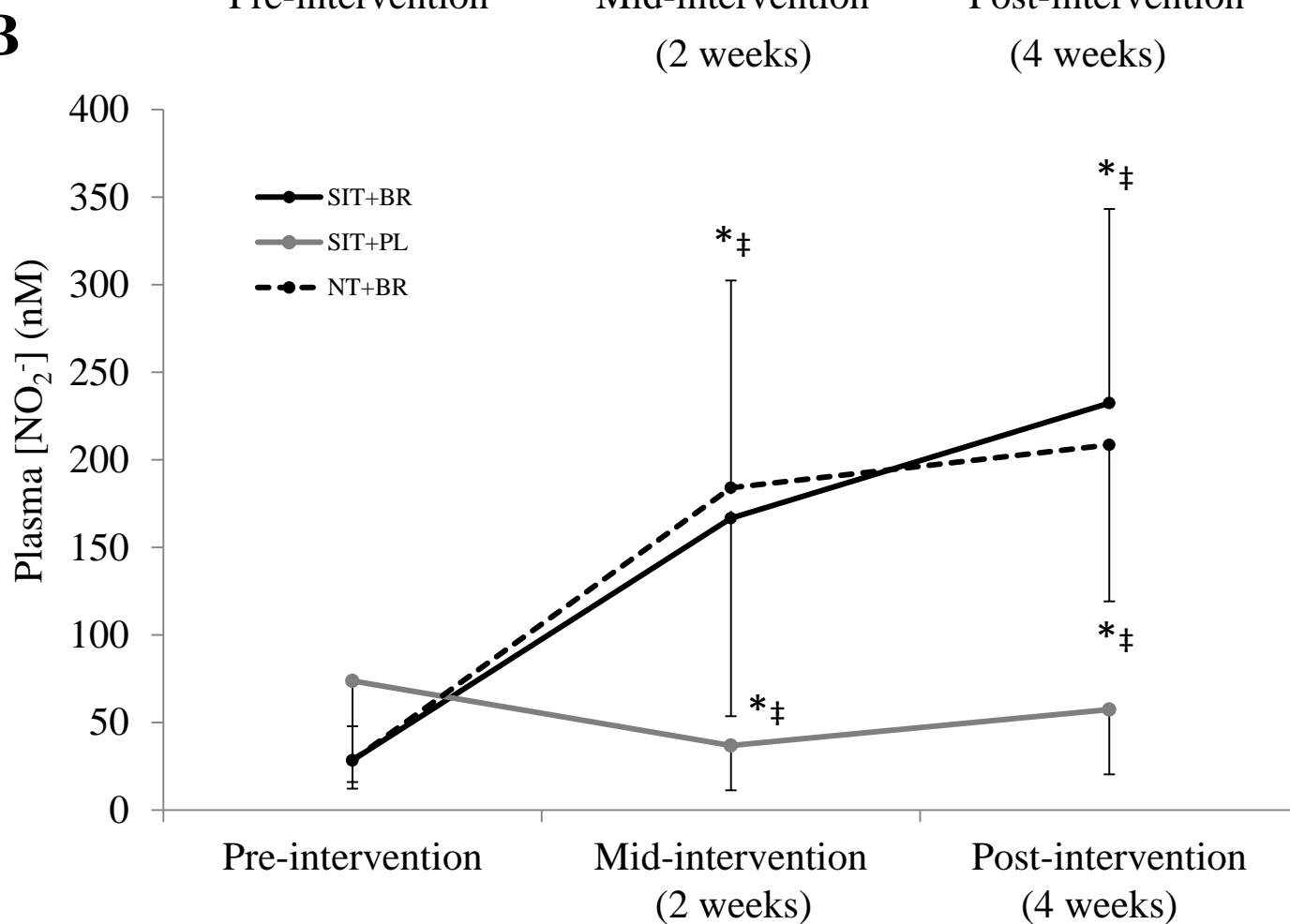
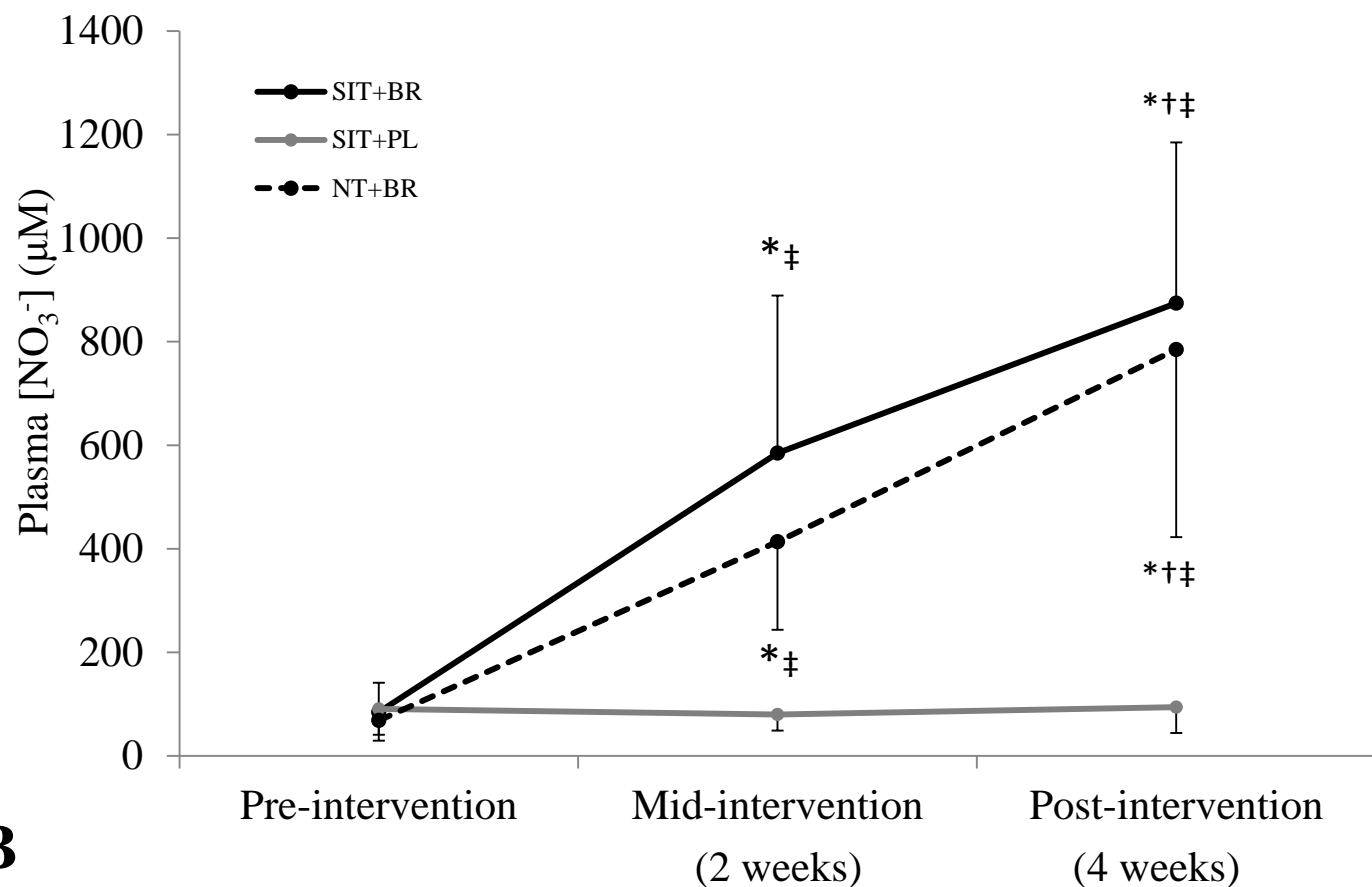


Figure 2

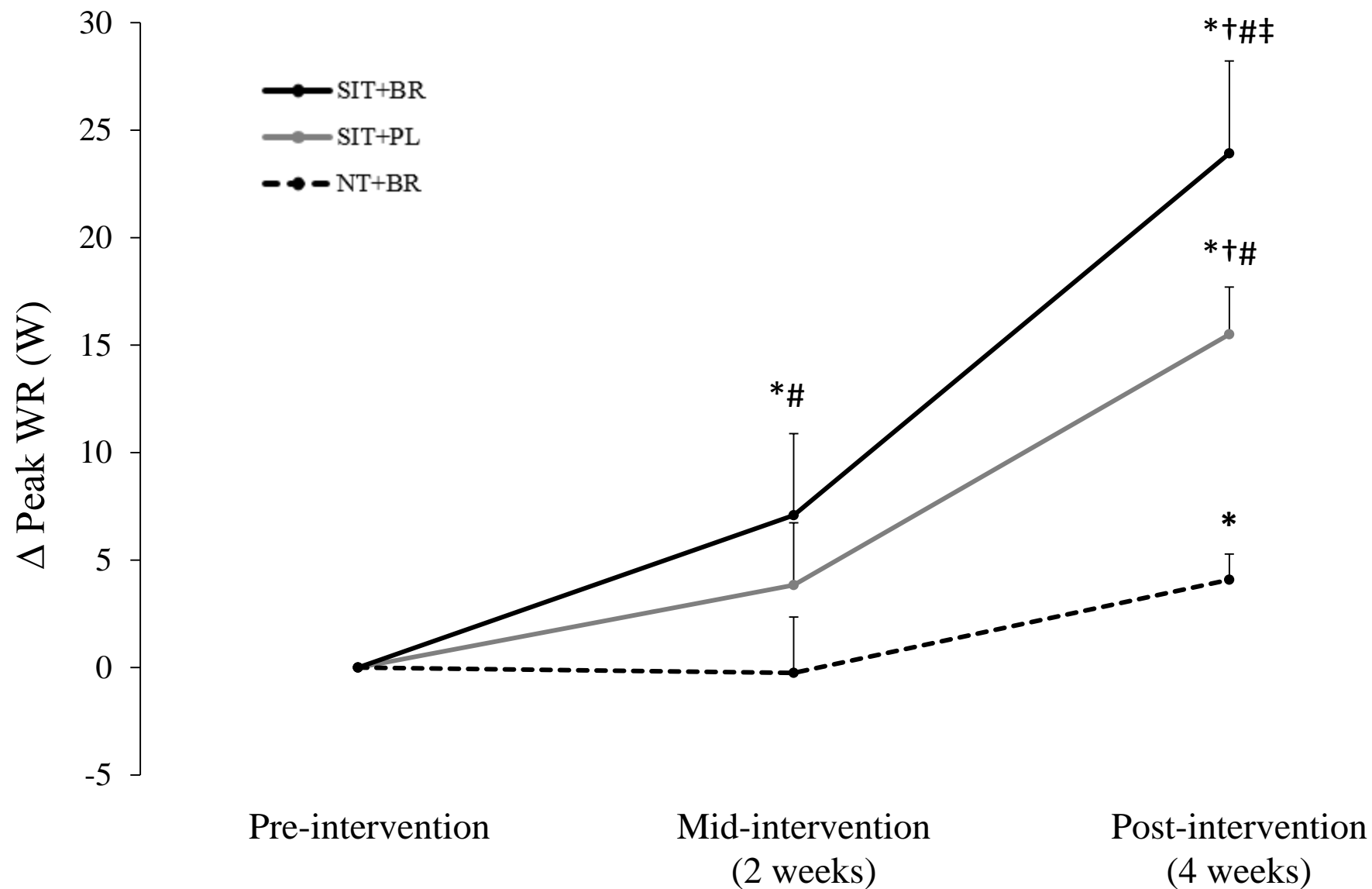


Figure 3

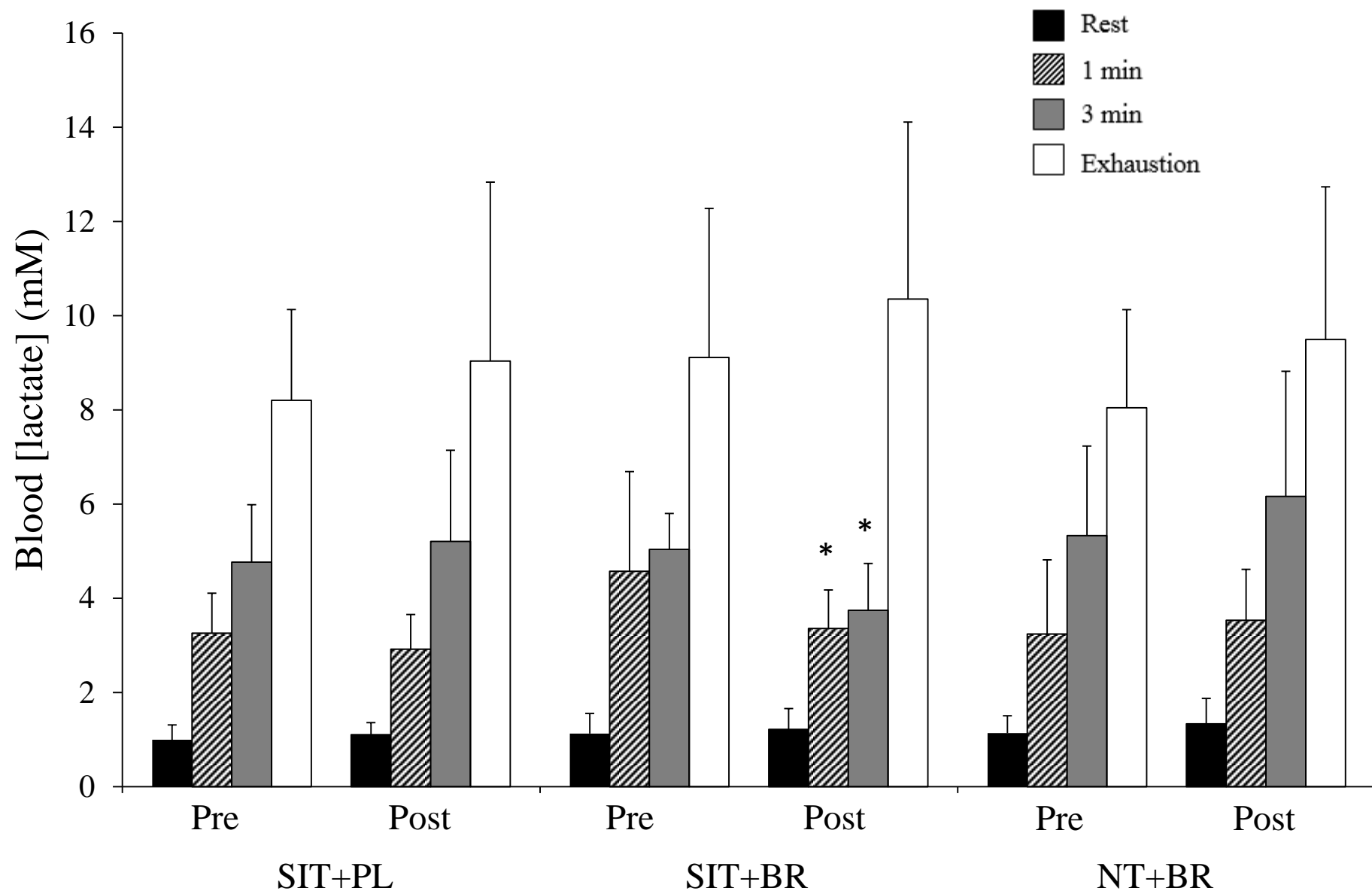


Figure 4

